REMARKS

Claims 13-16 and 18-32 are in the present application.

1. Rejections over Essenfeld, et al. under 102(e)

The Examiner repeated her rejection of Claims 13-16, 18, 25, 26 and 31 under 35 U.S.C. §102(e) as allegedly anticipated by Essenfeld, et al. Applicants respectfully submit that Essenfeld, et al, does not anticipate the presently claimed invention.

Claim 13 as currently amended in this response recites a DMSO concentration of greater than 20%. Essenfeld, et al., do not disclose a DMSO concentration of greater than 20%. Column 16, lines 13-45, cited in the Final Rejection, discloses a solution of 40% isopropyl alcohol, 40% acetone, 20% polyethylene glycol and 1% DMSO. In fact, every Example disclosed by Essenfeld, et al., teaches a DMSO concentration of only 1%. Accordingly, since Essenfeld, et al., does not disclose every element of the present claims, Applicants respectfully submit that the rejection under §102(e) should be withdrawn.

2. Rejections over Essenfeld under 35 U.S.C. §103(a)

The rejection over Essenfeld, et al., of Claims 19, 20, 22 and 32 under 35 U.S.C. §103(a) was maintained. Applicants respectfully submit that the present invention is not obvious over Essenfeld, et al.

Claim 20 has been cancelled. Claims 19, 22 and 32 remain pending.

Essenfeld, et al. teaches only DMSO concentrations of one percent (1%). Each of Examples 1 through 3 of Essenfeld, et al., teach use of DMSO at one percent (1%). In contrast, each of Claims 19, 22 and 32 of the present invention recite DMSO concentrations of greater than 20%. It is respectfully submitted that a concentration *twenty times (20x) greater* than that taught by Essenfeld is not obvious to one of ordinary skill in the art.

The Examiner stated that where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum by routine experimentation. However, the only general condition or range taught by Essenfeld is a single concentration of 1%. The rejected claims recite a concentration of 20% which lies 20-times out of that range.

Further, the prior art teaches away from increasing the DMSO concentration to greater than 20%. See "A Guide to the Properties and Uses of Detergents in Biology and Biochemistry," Judith Neugebauer, CALBIOCHEM Corporation (1988) (copy of relevant pages attached) (hereinafter referred to as "Neugebauer"). Essenfeld, et al., includes DMSO as a surfactant (see column 5, Line 24). Neugebauer teaches that detergents, which includes surfactants (see pg. 4), will solubilize membranes at detergent:protein ratios from 10:1 to 0.1:1 (see pg. 17). Thus, Neugebauer teaches that a DMSO:protein ratio of 0.1:1 will solubilize a tissue sample. Since Essenfeld, et al., considers preservation of morphology to be critical, a person of ordinary skill in the art would not have been motivated to increase DMSO concentration to 20%.

Neugebauer also teaches that some detergents will interfere with staining or fixing procedures (see pg. 16). Accordingly, since Essenfeld, et al., relates to fixation of tissues, a person of ordinary skill in the art would be motivated to minimize the amount of DMSO used. A person of ordinary skill in the art would not have been motivated to increase DMSO concentration 20-times.

Accordingly, it is respectfully submitted that the rejection of Claims 19, 20, 22 and 32 under 35 U.S.C. §103(a) should be withdrawn.

3. Rejections over Essenfeld in view of Evinger-Hodges under 35 U.S.C. §103(a)

Claims 27-30 were rejected under 35 U.S.C. §103(a) as being unpatentable over Essenfeld et al. in view of Evinger-Hodges, et al. (WO 90/02204). Applicant's traverse.

Claims 27-30 depend from Claim 13. Claim 13 recites a DMSO concentration of greater than 20%. As stated above, Essenfeld, et al., neither teaches nor suggests the presently claimed invention. Evinger-Hodges neither teaches nor otherwise suggests a DMSO concentration of greater than 20%. Accordingly, it is respectfully submitted that the rejection of Claims 27-30 under 35 U.S.C. §103(a) should be withdrawn.

4. Rejections over Essenfeld in view of Rogers under 35 U.S.C. §103(a)

Claims 21, 23 and 24 were rejected under 35 U.S.C. §103(a) as being unpatentable over Essenfeld et al. in view of Rogers. Applicant's traverse.

Claim 24 has been cancelled.

Claims 21 and 23 depend from Claim 13. Claim 13 recites a DMSO concentration of greater than 20%. As stated above, Essenfeld, et al., neither teaches nor suggests the presently claimed invention.

Applicants respectfully submit that it is Rogers is improperly cited as prior art. The Federal Circuit has held that "In order to rely on a reference as a basis for rejection of an applicant's invention, the reference must either be in the field of applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the invention was concerned." *In re* Oetike, 977 F.2d 1443, 1446 (Fed. Cir. 1992) Rogers describes a method to clarify and contrast intact biological tissue samples for microscopic analysis. Such a method is not in the field of the present Applicant's endeavor to stabilize nucleic acids. In fact, the USPTO classified Rogers as belonging to Class 435, Subclass 40.5. In contrast, the USPTO classified the present invention in Class 536. Thus, the USPTO patent classification system states that Rogers is not in the field of Applicant's endeavor.

Nor is Rogers reasonably pertinent. A person of ordinary skill in the art, interested in nucleic acid stabilization, would not logically look to a reference relating a method to clarify and contrast stain intact biological tissue samples for microscopic analysis. See *Wang Labs v. Toshiba Corp.*, 993 F.2d 858 (Fed. Cir. 1993) ("A reference is reasonably pertinent if, even though it may be in a different field from that of the inventor's endeavor, it is one which, because of the matter with which it deals, logically would have commended itself to an inventor's attention in considering his problem.") The solution cited by the Examiner is a fixative solution (for preparing cells for microscopic analysis). A person of ordinary skill in the art, interested in nucleic acid stabilization, would not logically consider fixative solutions for microscopy in considering an answer to her molecular problem.

Furthermore, the method of Rogers is not limited to the methanol and DMSO solution cited by the Examiner at Column 5 Line 50 through Column 6, Line 3. The method taught by Rogers also includes very low temperatures (-70°C), gentle agitation, application of a vacuum in

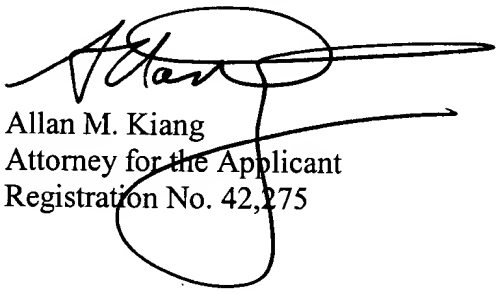
cycles, use of hydrogen peroxide, use of 100% methanol, use of pigment, use of a benzyl alcohol/benzyl benzoate solution, mounting, use of fresh BABB solution and viewing the specimen under a microscope. See Column 5, Line 62 through Column 6, Line 48. It is respectfully submitted that a person of ordinary skill in the art would not view this rather extensive method for viewing a specimen under a microscope as reasonably pertinent to her problem of nucleic acid stabilization. Nor would a person of ordinary skill, interested in nucleic acid stabilization, be motivated to focus upon a small portion of the overall non-analogous method taught by Rogers.

Accordingly, it is respectfully submitted that the rejection of Claims 21,23 and 24 under 35 U.S.C. §103(a) should be withdrawn.

CONCLUSIONS

The claims of the present application are believed to be in condition for allowance, and early notice thereof is respectfully requested.

Respectfully submitted,



Allan M. Kiang
Attorney for the Applicant
Registration No. 42,275

Becton Dickinson and Company
1 Becton Drive
Franklin Lakes, New Jersey 07416
(201) 847-7111

doc#62956

CALBIOCHEM® Biochemicals

A Guide to the Properties and Uses of Detergents in Biology and Biochemistry

By Judith Neugebauer
Clarkson University, Potsdam, New York

A 12-01-0

II. Physicochemical Properties

Detergent properties have been the subject of several reviews (1-5, 37, 38, 45, 48, 59), the most recent of which appeared in 1984. The information given here is largely a synopsis of this material, with some points more strongly emphasized, clarified or updated.

Detergents are known also as tensides (1), soluble amphiphiles (2), soaps (usually restricted to the alkali metal salts of long-chain fatty acids) and surfactants. Perhaps the most descriptive of these, in terms of definition, is the word surfactant, which is a contraction of the phrase "surface-active agent." Chemical compounds that exhibit surface activity—or more generally, interfacial activity—migrate to the interface(s) when placed in solution. This migration results in a depression in the surface tension (or interfacial tension) of the solution as compared to the surface tension of the pure solvent.

More than simply migrate, however, surface-active molecules, by virtue of their "dual nature" (10) actually align at the interface(s). Each detergent has a *hydrophobic* portion, which is more soluble in oil or other hydrocarbon solvents, and a *hydrophilic* portion, which is more soluble in water. Their alignment at interfaces (hydrophobic tails in the air or in the hydrocarbon phase and hydrophilic heads in the water) reflects the tendency of surfactant molecules to assume the most energetically favored orientation. Tanford (10) has discussed this phenomenon in detail.

Surfactants also exhibit self-aggregation. At low concentration in aqueous solution, detergents exist as *monomers* (single, unclustered detergent molecules). Above a characteristic limit, called the *critical micellization concentration* (CMC), *micelles* or clusters of detergent molecules are formed. Micellization is also a consequence of the dual nature of detergent molecules. Figure 1a shows the generalized structure of a surfactant micelle in aqueous solution (6). The detergent molecules are organized in such a way that the hydrophobic portions are in contact with each other in the micellar "core" and the hydrophilic portions form a "shell" in contact with the aqueous environment. The number of monomers that come together to form a micelle is called the *aggregation number* (N).

4. How will the detergent affect the rest of the system?
   - If proteins are part of the system will they be denatured by the detergent?

     If denaturation is strictly defined as unfolding of native protein structure, then detergents with long (>C_{12}) unbranched hydrocarbon chains are usually considered to be the most denaturing. It has been proposed that this is because long, flexible chains can best "reach in" to disrupt a folded protein structure (35). However, if denaturation is defined as any process through which full native activity is lost, then practically any detergent can be denaturing under the wrong conditions. In other words, it can never be assumed that a detergent will not harm protein structure. Assay of protein activity in solubilized preparations compared to that of the protein in its native state is an essential indication of denaturation during solubilization. Note that even if a protein is denatured, the effect may be reversible if the detergent is removed in a final step. This occasionally applies even to SDS, the quintessential denaturing detergent (131).

PRESERVING STRUCTURE IS NOT CRITICAL FOR US, WE LYSE THE CELLS ANYWAY. FOR ESSENFELD STRUCTURE IS CRITICAL "SURFACTANT" CONC. CANNOT BE TOO HIGH.

5. How much detergent should be used?

   Optimal detergent concentration must be determined experimentally for each system. However, Hjelmeland, et al. provide guidelines to use to establish detergent:protein ratios for the initial solubilization trial (38). They suggest that detergent:protein (w:w) ratios covering the range 10:1 to 0.1:1 be tested. An alternative guideline provided by Reynolds allows one micelle for every 5-10 lipid molecules plus 6-11 micelles for each protein molecule (48). The dispersion of lipid-free proteins into one functioning unit per micelle can occur under these conditions.

THESE ARE SOLUBILIZING LEVELS

6. Depending on the application, other questions such as the following may become relevant:
   - What is the toxicity?

     Digitonin is a cardiac glycoside and must be handled with care..
   - What is the cost?

     $C_{12}E_9$ is a monodisperse, high quality, relatively expensive detergent. THESIT or LUBROL PX are commercial, polydisperse near-equivalents that might be acceptable substitutes, with particular economic advantage in larger scale experiments.
   - Will the detergent be affected by the system?

     The alkyl glucosides and maltosides should not be used in systems that contain glycosidase enzymes.